Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 Spl Issue III 2014: 14-19

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

Full Length Article

Investigation of adhesion and survival of probiotic bacteria on Iranian fermented green olives during cold storage

Romina Farajpour[1], Mahnaz Hashemiravan*[1],Fatemeh Noorbakhsh[2]
[1]Department of Food Science and Technology, Islamic Azad University , Varamin-pishva Branch ,Varamin-Iran
[2] Department of Biology – Microbiology, Islamic Azad University , Varamin-pishva Branch ,Varamin-Iran

ABSTRACT
The olives and probiotic foods of the invention are an effective means to treat or prevent intestinal disorders or restore the intestinal flora after antibiotic therapy. The aim of this studywas to investigate adhesion and viability of probiotic bacteria (Lactobacillus acidophilus and Lactobacillus casei separately and a blend of them) on olive and physicochemical and sensory charactristics of probiotic fermented olive during 90 days storage in 4 ^{0}C .Results indicated thatthe number of live probiotic bacteria on the surface of the sample olive containing Lactobacillus acidophilus for 45 days and all the samples in brine for 60 days was higher than the standard amount (10^{6}cfu/ml). Gradually, it was decreased significantly ($p<0.05$) and at the end of storage period, the olive sample containing Lactobacillus acidophilus had the highest survival. Acidity and pH of the samples were significantly ($p<0.05$) increased and decreased respectively.
Keywords*: green olive, probiotic, Lactobacillus acidophilus, Lactobacillus casei, fermentation, during cold storage*

Received 28.04.2014 Revised 19.05.2014 Accepted 11.06. 2014

INTRODUCTION

Consumption of probiotic bacteria plays an important role in preservation of the host' health. Probiotic bacteria have some advantages including modulating gut bacteria, producing antimicrobial substances, stimulating the immune response, partial inhibition of enzyme activity, production of short chain of fatty acids which moderate gut acidity [18, 19]. To realize stability of these health effects, regular consumption of 10^6 to 10^9 live cells is recommended. *Lactobacillus acidophilus*is one of the most common bacteria species from which many probiotic strains have been isolated. Probiotics are live microbes of which regular and sufficient consumption will have beneficial effects for the consumer's health [11]. Lactobacillus casei is
another important probiotic in nutritional products. This bacteria is gram-positive, mesophile, rod-shaped, microaerophilic, catalase-negative and non-spore [3]. In the recent decade, development and consumption of probiotic foods have been increased significantly parallel with awareness about beneficial effects of these foods on the health of the gut and preventing the diseases [20]. Effectiveness of probiotic foods depends on proper survival of the mentioned microbes until the time of food consumption and that, after digestion and entering the digestive system, withstand the acidic conditions of the stomach and the presence of bile in the duodenum, ultimately they reach the gut by sufficient number. Therefore, the residuals of these microbes in the carrier nutrient are very important during its production and storage [22]. Nowadays, food industry has focused on to generating new non-dairy foods which can provide probiotic regular distribution in the people with lactose intolerance or those with limited consumption of dairy products [5]. Olive is an appropriate environment for microbial population survival particularly lactic bacteria due to having almost all essential and nutrients [13, 16].Added bacteria improving the health of olive increases nutritional trait of olive including: A) Antioxidant polyphenols which have strong activity in trapping free radicals and preventing the atherogenesis. B) Minerals including potassium, magnesium, manganese, iron, calcium, vanadium and sulfur (14-38 mg/100g) which is an essential element for sulfurization metabolism of protein [10]. C) Vitamins (belonged to the groups A, B, E) which play a role in postponing the cell senescence[9, 10]. D) Oil section

(14-30%) which mostly includes single-band unsaturated fatty acids that are effective in increase of protective cholesterol HDL level [24]. Lactic acid bacteria are effective in development of self-fermentation or start of lactic fermentation of edible olives and cause to improve sensorial traits [8]. Olive and new probiotic foods are the most effective medium to treat or prevent intestinal diseases or restore intestinal flora after antibiotic therapy [21]. Preparation of probiotic vegetables such as probiotic olive causes to attract customers more. By the way, olive provides an appropriate bed for probiotics transition in terms of structure and nutritional traits [14, 23]. In contrast to this review, the scientists have reported that, although probiotic products production with high population f probiotic bacteria cells is beneficial, some sensorial traits of probiotic products are not desirable in the view of consumers [12]. In this study, adhesion and viability of probiotic bacteria (*Lactobacillus acidophilus*and *Lactobacillus casei* separately and a blend of them) on olive and physicochemical and sensory characteristics of probiotic fermented olive during 90 days storage in 4 ^{0}C were investigated.

MATERIALS AND METHODS

Bacterial strain and culture conditions

Pure strains of *Lactobacillus acidophilus*(LA-5) and *Lactobacillus casei*(LC-01) were suppliedbyChr-Hansen (Denmark). The considered bacteria were cultured in the mediumof MRS broth and were put in an incubatorat 37 ^{0}C for 24 h. Then, 200 microliter of the medium containing bacteria was blended with 800 microliter of sterile glycerol and stored in -20 ^{0}C. This culture was used to new discovery of bacteria. In order to obtain single colonies, active cultures of *Lactobacillus acidophilus* and *Lactobacillus casei*, linear culture was conducted in some plates containing solid sterile MRS and was stored at 37 ^{0}C for 24 h.

Preparation of bacterial inocula for olive fermentation

The activated culture was inoculated by three loops to 100 ml of sterile MRS broth medium and then, it was kept at 37 ^{0}C for 24 h. In order to isolate biomass of the medium containing bacteria, it was taken to a falcon and was centrifuged by 3000 rpm for 10 minutes. The collected cells were diluted (10^{13} CFU/ml) by sterile distilled water. In this regard, a spectrophotometry instrument (Milton Roy Company,USA) with wavelength of 623 nm was used by McFarland method [2]. Considering that, the used bacterial population in this study was 10^{13}, so, OD=2.44 in wavelength of 623 nm.

Olive preparation

The olives used in this research, were from yellow variety, Roudbar region which were washed, sorted and graded after harvesting and isolation of tail, leaves and branches; then,olives were treated for 4h with a NaOH solution(0.2normal) to remove oleuropein and bitterness. In order to remove NaOH solution the olives were immersed in water for 72 h. Then, the olives were put in brine(7%wt/volNaCl)for 20 days and ultimately, they were put in water for 4 days to remove the salts from the olives' texture. After that, the olives were put in brine and were pasteurized to eliminate natural organisms completely.

Brine preparation

Pure salt(NaCl) was bought from Dr. Mojallali chemical lab, and sucrose was purchased from Sugar FarmandCompany. In order to produce brine, distilled water along with salt(4%wt/volNaCl) and sucrose(0.5%wt/vol) were used. Then, the olives were added under sterile conditions.

Probiotic fermented olive manufacture

10 olives without bitterness were added to 100 ml autoclaved under completely sterile condition brine, then, 10 ml suspension of *Lactobacillus acidophilus* was inoculated the first olive sample. 10 ml *Lactobacillus casei* was added to the second olive sample, and *Lactobacillus acidophilus* and *Lactobacillus casei* were added to the third olive sample as starter. Finally, the olive samples were stored in 4^{0}C and were sampled at the days 3, 15, 30, 45, 60, 90.

Microbial analysis

In order to count the bacteria existing on olives,three olives from the main sample were transmitted to the 50 ml tube containing 20 ml sterile NaCl(0.85%,wt/vol) solution supplemented withTween 80(0.025%,vol/vol), and shaken vigorously for 1h.Then a proper dilution was provided from each sample. In order to count bacteria, pour plate technique,MRS agar medium (Merck, Germany) were used. The plates were incubated aerobically at 37^{0}C for 24h.

In order to count bacteria in the brine, pour plate technique andMRS agar medium (Merck, Germany) were used.The plates were incubated aerobically at 37^{0}C for 24h [14].

Physicochemical analyses

Calculation of pH was conducted according to Iran National Standard No.987. pH meter (MA235,HANNA,Milan,Italy) was adjusted by buffered solutions and then, the sample was thrown in a 100 ml beaker and its pH was measured under 25^{0}C [1].

Percentage of acidity was calculated according to the guideline of Iran National Standard No.987.20 ml of the filtered solution was thrown in a marked flask and was tittered and then, percentage of acid was computed using the Equation (1).

$$Acidity\ percentage = \frac{0.009 \cdot 100 \cdot N}{S}$$ Equation (1)

Where

S=sample volume (ml)

N=the used sodium hydroxide (ml)

Sensory evaluation

In order to evaluate sensorial characteristics of the samples, 7 trained evaluator were used by scoring method so that flavor, odor, color, texture and structure of the products produced from olive were evaluated at the day 90 after production and storage in 4^0C. The evaluators were asked to score 5 for excellent, 4 for very good, 3 for good, 2 for moderate and 1 for weak. After scoring, the given scores were multiplied by the coefficient of each factor and finally, the score of each characterictic was calculated. Then, the summation of the scores of analyzed factor was considered as total score. The scores given to flavor, odor and texture were multiplied by 3, 5, 2 respectively [7].

Statistical analysis

For statistical analysis of the considered characteristics,generalized linear models with measures repeated over the time were used and the means of treatments were compared by Duncan method and ANOVA. All tests were conducted in three replications, and their significant difference was evaluated at probability level of 0.05. Two-way variance analysis and Duncan test were used to compare the means. Statistical graphs were drawn by Excel software and all statistical computations were conducted using SPSS 18 software.

RESULTS

1. survival of probiotic bacteria

The number of probiotic bacteria on olives has been shown in Figure 1. Bacterial population showed a significant increase in all samples until the 15th day ($p<0.05$). After the day 15, a significant reduction was observed in all the samples ($p<0.05$). By continuing this descending trend, the highest bacterial population was observed in *Lactobacillus acidophilus* after 3 months storage which had a significant difference to other samples ($p<0.05$).

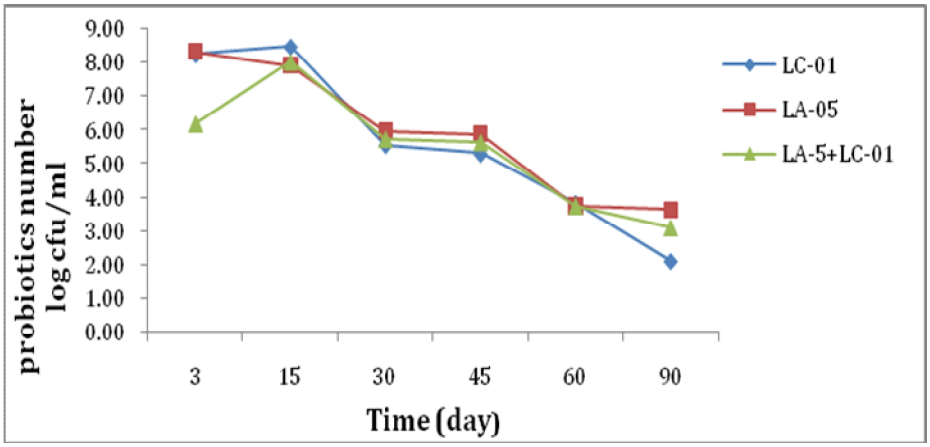

Fig. 1. Changes of number of probiotic bacteriaon olives during refrigerated storage. *Lactobacillus casei* (LC-01), *Lactobacillus acidophilus* (LA-5) mixture of *Lactobacillus acidophilus* and *Lactobacillus casei*(ratio: 50/50) (LA-5 + LC-01).

Number of probiotic bacteria in brine has been shown in Figure 2. Bacterial survival until the 60th day in all samples was much higher than the recommended minimum population to achieve beneficial properties (10^6cfu/ml). After the 60th day, a significant reduction was observed in all the samples ($p<0.05$). At the end of storage period, the highest bacterial survival was observed in *Lactobacillus acidophilus* sample with population of 10^6cfu/ml which had a significant difference to the other samples ($p<0.05$).

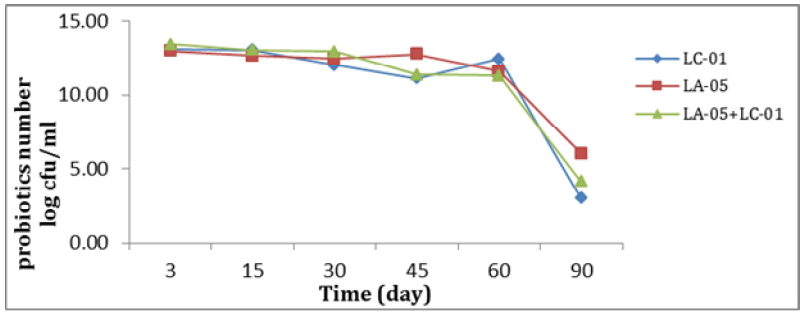

Fig. 2. Changes of number of probiotic bacteriain brine during refrigerated storage.*Lactobacillus casei* (LC-01), *Lactobacillus acidophilus* (LA-5) mixture of *Lactobacillus acidophilus* and *Lactobacillus casei* ratio (ratio: 50/50) (LA-5 + LC-01).

pH

The values of pH in the samples' brine has been shown in Figure 3. Adding bacterial species to the samplescaused to reduce pH significantly compared to the initial pH (5.9±0.0) ($p<0.05$). Drop in pH occurred by 2 units since the first day until the third day (variation range: 3.87±0.03 to 3.73±0.03). The lowest value of pH was observed in the sample containing a mixture of two bacteria which had a significant difference to the other samples ($p<0.05$).

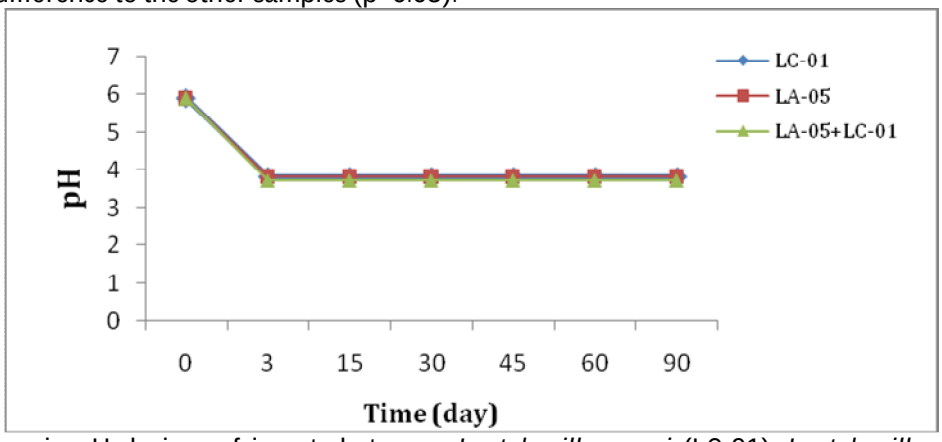

Fig. 3.changes in pH during refrigerated storage. *Lactobacillus casei* (LC-01), *Lactobacillus acidophilus* (LA-5) mixture of *Lactobacillus acidophilus* and *Lactobacillus casei* ratio (ratio: 50/50) (LA-5 + LC-01).

Acidity

The acidity values has been shown in Figure 4. Increase of acidity was significant during the early three days of fermentation in all the three samples compared to the initial acidity (0.022± 0.0) (variation range: 0.15± 0.003 to 0.17± 0.003).At the third day, the maximum amount of acidity was observed in the sample containing a mixture of two bacteria which had a significant difference to the other samples ($p<0.05$). After the third day, the amount of acidity had no significant difference ($p>0.05$).

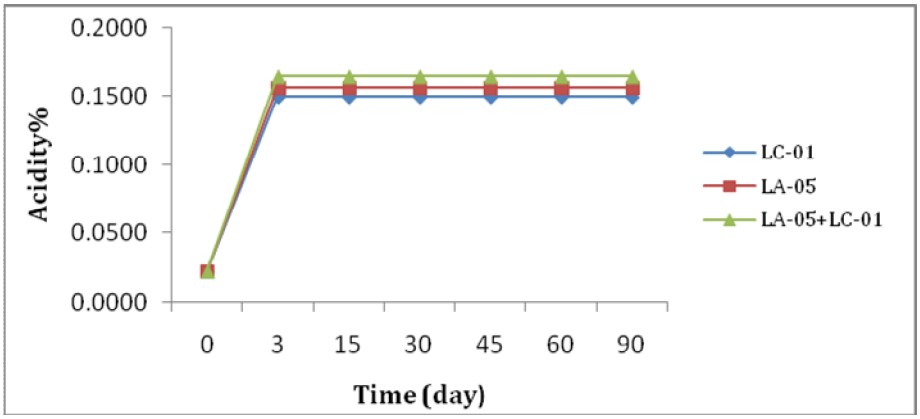

Fig.4.changes inacidityduring refrigerated storage. *Lactobacillus casei* (LC-01), *Lactobacillus acidophilus* (LA-5) mixture of *Lactobacillus acidophilus* and *Lactobacillus casei* ratio (ratio: 50/50) (LA-5 + LC-01).

Sensory evaluation

Table 1 demonstrates sensory characteristics of the samples at the 90 days. The highestflavor score was for sample containing Lactobacillus casei which had a significant difference to the other samples ($p<0.05$).The sample containing Lactobacillus casei had the highestodor score and showed no significant difference to the other samples. There was no significant difference between the color score and texture score of the samples ($p>0.05$). The highest total score was for sample containing Lactobacillus casei which had a significant difference to the other samples ($p<0.05$).

Table 1. Comparison of sensorial indices of probiotic fermented olive samples at the day 90in 4 ^{0}C.

Total score	Texture	Color	Odor	Flavor	Time (day) Sample
3.86±016^a	8.86±0.40a	10±0	10.29±0.60^a	17.14±1.48^a	Lactobacillus casei
3.57±0.14^b	8.86±0.40^a	10±0^a	9±0.92^a	15±1.09^b	Lactobacillus acidophilus
3.57±0.14^b	8.86±0.40^a	10±0^a	9±0.92^a	15±1.09^b	Lactobacillus casei+ Lactobacillus acidophilus

Different letters represent significant statistical difference at probability level of $p<0.05$.

DISCUSSION

Olive is an appropriate environment for microbial population survival particularly lactic bacteria due to having almost all essential and nutrients [2]. Also several reports have shown that, olive as probiotic carrier causes to increase bacterial survival (durability time by 90 days) and the number of bacteria population (10^7cfu/ml) compared to the probiotic products based on milk. In these products, durability time is about 30 days and bacterial population is 10^6cfu/ml [15]. Hence, some researchers have studied the survival of some probiotic bacteria on various variety of olive. Lavermicocccae*t al*. (2005) investigated bacterial survival of *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Bifidobacterium* during 90 days where the best bacteria survival on the olive level and brine was for the sample which had been inoculated with *Lactobacillus paracasei* [14]. According to Fig. 3, the maximum drop of pH compared to the initial pH in all samples has occurred during the early three days; while, the drop in pH has not been tangible after the third day and the reduction trend of pH remains constant which can be due to rapid fermentation process during the early days. Significant reduction of pH at the early days of fermentation has been reported by some researchers. De Belli *et al*. (2010)observed a rapid drop of pH during the early two days of fermentation in the samples inoculated with *Lactobacillus paracasei* [6]. Panagou*et al*. (2007) used the strains of *Lactobacillus pentosus*and *Lactobacillus Plantarium*, and found that, in the samples inoculated by these strains, pH reached its minimum amount at the fifth day and then, it remained relatively constant [17]. According to Fig. 4. The maximum increase of acidity occurs at the early days (until the third day, and after that, the increase of acidity remains constant and has no significant change which can be due to the increase of bacterial population (because of bacteria adaption to the new environment and reaching the logarithmic phase) at the beginning of fermentation period. However, some other factors such as temperature and the amount of consumed sugar, etc are also effective. Panagou et al. used the strains of *Lactobacillus pentosus* and *Lactobacillus Plantarium*, and found that, in the samples inoculated by these strains, acidity reached its minimum amount at the 7th day and then, it remained relatively constant [17]. According to Table (1), the maximum total score is for sample containing *Lactobacillus casei* which represents appropriateness of this samples compared to the two others. It should be mentioned that, olive fermentation by these bacteria species had no impact on the fruit color.

Consequently, according to the results of this research, *Lactobacillus acidophilus* had a better adhesion and survival on olives as well as in brine during 90 days in 4 ^{0}C, compared to Lactobacillus casei. Also, olive can be used as a proper carrier with long durability to carry the useful bacteria and delivering to the consumer. In order to continue the work and the use of results of this study, it is suggested that, clinical investigation of the strains' survival in the consumers to be conducted in addition to the use of other probiotic strains and investigating the survival and characteristics of the product. Also, effectiveness of the use of product on the consumer's product should be studied.

ACKNOWLEDGEMENT

Hereby, the laboratory staffof Health Department of University of Tehran and research deputy of Islamic Azad University of Varamin - Pishvaare appreciated for great cooperation to do this study.

REFERENCES

1. National standard of Iran, No. (1987), Processed olives - Specifications and Test Methods, Standards and Industrial Research Institute of Iran.
2. Ashrafi, F. (2006). Practical microbiology, 1stEdition, Ahsan Press: 173-180.
3. Mortazavian, A. M. And Sohrabvandi, S.(2006). A review on the properties of probiotics and probiotic food products, 1st Edition, Tehran, Eta Press: 25-32.
4. Carluccio,M.A., L,Siculella., M.A.,Ancora., M,Massaro., E,Scoditti, C,Storelli., F,Visiolo. A,Distante.,R.DeCaterina,. (2003). Olive oil and red wine antioxidant polyphenol inhibit endothelial activation. Arterioscler. Thromb. Vasc.Biol. 23,622-629.
5. Charalampopoulos,D.,R.,Wang,S.S.Pandiella,andC.Webb. (2002). Application of Cereals and Cereal components in Functional Food:areview.Int.J.Food Microbiol.79:131-141.
6. DeBellis,P .,Valerio ,F. ,Sisto ,A., Lonigro , S .L. , Lavermicocca , P . (2010). Probiotic table olive:Microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei IMPC2.1* in a industrial plant.International Journal of Food Microbiology140,6-13.
7. Fedela,C.,Abderrahim,C.Ahmed,B. (2009)..Physico-chemical and rheological properties of yogurt manufactured with ewels milk and skim milk.African Journal of Biotechnology,8(9),1938-1942
8. Gardiner , G., R.P, Ross, J.K. Collins,G. Fitzgerald ,C. Stanton . (1998). Development of aprobiotic cheddar cheese containing human-derived *Lactobacillus paracasei* strains . Appl . Environ.Microbiol.64.2192-2199.
9. Garrido Fernadez,A.,M.J.FernadezDiez, and M.R.Adams. (1997). Physical and chemical characteristics of the olive fruit, p.67-109. In A. Garrido Fernandez et al.(ed),Table olive. Chapman & Hall, London, United Kingdom.
10. Giomo,A. (2001). Dossier olive da mensa. Unarisorsa da scoprire. Olive oilo 9:20-23.
11. Goktepe,I.,V.K.Juneja and M.Ahmenda. (2005)..Probiotic in Food Safety and Human Health,Isted.,CRC Taylor & Francis Group,USA.
12. Kirjarainen,P.V.,Arvola,T.,Salminen,SJ. (2002)..Aberrant Composition of gut microbiota of allergic infants: a target of antibacterial the rapid at weaning, Gut. 51-55.
13. Lavermicocca,P.,M.Gobbetti,A.Corsetti,andL.caputo. (1998). characterization of lacic acid bacteria isolated from olive Phylloplane and table olive brines. Ital.J.FoodSci.10.27-39.
14. Lavermicocca,P.,Valerio,F.,Lonigro,S.,Angelis,M.,Morelli,L.,Callegari,M.,Rizzello,C.,Visconti,A.(2005). Study of adhesion and survival of *lactobacilli* and*bifidobacteria* on table olive with the aim of formulating a new probiotic food.
15. Lourens-Hattingh,A.,and B.C.Viljoen. (2001). Yogurt as probiotic carrier food.Int.Dairy J.11:1-17.
16. Nychas G.-H.E.,E.Z.Panagou,M.L.Parker,K.W.Waldron, and C.C.Tassou. (2002). Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine.Lett. Appl. Microbiol.34:173-177.
17. Panagou , E.Z. , Schillingr , U. , Franz , C.M.A.P. , Nychas , CJ.E . (2007). Microbilogical and biochemical profile of CV. Conservolea naturally blank olive during controlled fermentation with selected strains of lactic and bacteria.Food Microbiology25,340-358.
18. Perdigon,G.De Macicas,M.E.Alvarez,S.Oliver,G.DeRuiz,H. (1998). Systemic augmentation of the immune response in mice by feeding fermented milk with *Lactobacillus acidophilus* and *Lactobacillus casei*.Immunology 63,17-23.
19. Picard,C. Fioramonti,J. Francois,A. Robinson, T.Neant,F. Matuchansky,C . (2005). Review article: Bifidobacteria as probiotic a gents-physiologyical effects and clinical benfits . Alimentary pharmacology and Therapeutics 22,495-572.
20. Saarela,M.,L. Lahteenmaki , R ., Crittendn , S . Salminen , T.Mattila-Sandholm . (2002). Gut bacteria and health foods – the European perspective.INT.J.Food Microbiol.78:99-117.
21. Sabatini,N. (2009). Recent patents concerning organoleptic and Healthy properties of table olive.Recent Patents on chemical Engineering2,37-42.
22. Saxelin,M.,Grenov,B.,Svensson,U.,Fonden,R.,Reniero,R., Mattila-Sandholm,T. (1999). The technology of probiotics.Trends in Food Science &Technology,10,387-392.
23. Valerio, F.Debellis,P.Lonigro,S.L.,Monrelli,L.Visconti,A.Lavermicocca,P. (2006). In vitro and in vivo survival and transit tolerance of potentially probotic strains carried by artichokes in the gastrointestinal tract.Applied and Environmental Microbiology 72,3042-3045.
24. Visioli,F.,A.Poli, and C. Galli. (2002). Antioxidant and other biological activities of phenols from olives and olive oil.Med.Res.Rev.22:65-75.